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(54) Title: BRADYKININ ANTAGONIST PEPTIDES CONTAINING INDANE-SUBSTITUTED AMINO ACIDS

(57) Abstract

The present invention pertains to modified bradykinin antagonist peptides that contain indane substituted amino acids.

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BRADYKININ ANTAGONIST PEPTIDES CONTAINING INDANE-SUBSTITUTED AMINO ACIDS

BACKGROUND OF THE INVENTION

The invention relates to novel biologically active peptides which act as antagonists of the biological activities of bradykinin and its homologs and congeners, the pharmaceutically acceptable salts of these antagonists, and their application as therapeutic agents.

Bradykinin (BK), a nonapeptide (Arg^1-Pro^2-Pro^3-Gly^4-Phe^5-Ser^6-Pro^7-Phe^8-Arg^9) and its physiologically important related peptides, kallidin (Lys-bradykinin) and Met-Lys-bradykinin, exhibit physiological actions which qualify them as mediators of inflammatory reactions, hypotensive states, and pain. Bradykinin is overproduced in pathological conditions such as septic (Robinson et al., *Am. J. Med.* 59: 61 (1975)) and hemorrhagic (Hirsch et al., *J. Surg. Res.* 17: 147 (1974)) shock, anaphylaxis (Collier and James, *J. Physiol.* 160: 15P (1966)), arthritis (Jasani et al., *Ann. Rheum. Dis.* 28: 497 (1969); Hamberg et al., *Agents Actions* 8: 50(1978); Sharma et al., *Arch. Int. Pharmacodyn.* 262: 279 (1983)), rhinitis (Proud et al., *J. Clin. Invest.* 72: 1678 (1983); Naclerio et al., *Clin. Res.* 33: 613A (1985)), asthma (Christiansen et al., *J. Clin. Invest.* 79: 188

(1987)), inflammatory bowel disease (Zeitlin and Smith, *Gut* 14: 133 (1973)), and certain other conditions including acute pancreatitis, post-gastrectomy dumping syndrome, carcinoid syndrome, migraine, and hereditary angioedema (Leme, Handb. Exp. Pharmacol. 50/I: 464 (1978)). The production of bradykinin results in pain at the site of the pathological condition, and the overproduction intensifies the pain directly or via stimulation by bradykinin of the activation of the arachidonic acid pathway which produces prostaglandins and leukotrienes, more distal mediators of inflammation (Handbook of Experimental Pharmacology, Vol. 25, Springer-Verlag (1969), and Vol. 25 Supplement (1979); Stewart, in "Mediators of the Inflammatory Process," Henson and Murphy, eds., Elsevier, (1989)).

Bradykinin has been found to be produced in inflammatory reactions in the intestine, provoking contraction of smooth muscle and secretion of fluid and ions. The existence of specific bradykinin receptors in the mucosal lining of the intestine and in intestinal smooth muscle is demonstrated by Manning et al. (*Nature* 229: 256 (1982)), showing the influence of bradykinin in very low concentrations upon fluid and ion secretion.

The production of bradykinin and associated pain in angina has been studied and reported (Kimura et al., *Amer. Heart J.* 85: 635 (1973); Staszewska-Barczak et al., *Cardiovasc. Res.* 10: 314 (1976)). The reported action of bradykinin and prostaglandins acting in concert are the natural stimulus for excitation of the sensory receptors signalling the pain of myocardial ischemia.

Bradykinin and bradykinin-related kinins are not only produced endogenously, but may also be injected into an animal via stings or bites. It is known that insects such as hornets and wasps inject bradykinin related peptides that cause pain, swelling and inflammation.

Bradykinin and related peptides exert their actions on biological systems by combining with specific receptors on cell membranes in the affected tissues. These receptors are of two classes, designated B1 and B2. The B2 receptors require the entire bradykinin sequence for effective receptor combination and production of the biological effects, whereas the B1 receptors do not respond to intact bradykinin, but respond selectively to bradykinin lacking the carboxy-terminal arginine residue; this peptide is designated [des-Arg^9]-bradykinin. [des-Arg^9]-Bradykinin is produced in the body by one of the enzymes that normally destroys bradykinin, the plasma enzyme carboxypeptidase N, that removes the carboxy-terminal arginine residue. Essentially all normal physiological responses and many pathophysiological responses to bradykinin are mediated by B2 receptors, whereas in certain damaged tissues and in certain kinds of chronic inflammation, B1 receptors are induced. The currently accepted wisdom is that bradykinin antagonist drugs for treatment of chronic inflammation must have antagonist action at both B1 and B2 receptors.

The search for understanding of the mechanisms of action of bradykinin, which is essential for the development of useful tools for diagnostic use, and for the development of therapeutic agents aimed at alleviating the intense pain and other symptoms caused by the overproduction of bradykinin, was severely

hindered by the lack of specific sequence-related competitive antagonists of bradykinin until the discovery of the first effective bradykinin antagonists by Vavrek and Stewart in 1985 (Vavrek et al., *Peptides* 6:161-164 (1985); U. S. Patent 4,693,993). In these early antagonists, the proline residue at position 7 of bradykinin was replaced by a D-aromatic amino acid residue, usually D-phenylalanine or D-thienylalanine. Subsequently, many modifications of the original bradykinin antagonists have been described (reviewed by J. M. Stewart and R. J. Vavrek in R. M. Burch, ed., "Bradykinin Antagonists," Pergamon, 1990), but most effective antagonists have had an aromatic amino acid residue at positions 5 and 8 and a D-aromatic residue at position 7. In certain antagonists, positions 5, 7, and 8 are occupied by aliphatic amino acid residues (J. M. Stewart, et al. in "Peptides 1992," C.H. Schneider and A.N. Eberle, eds., ESCOM, Leiden, 1993, pp 691-692).

Antagonists for bradykinin B1 receptors are obtained by replacing the phenylalanine residue at position 8 of [des-Arg^9]-bradykinin by an aliphatic amino acid, such as leucine. Thus, [Leu^8, des-Arg^9]-bradykinin and Lys-[Leu^8, des-Arg^9]-bradykinin are effective B1 receptor antagonists. All of the bradykinin antagonists of the type described by Stewart and Vavrek, containing a D-amino acid residue at position 7, act only upon B2 receptors. In certain *in vivo* assays, some of these early antagonists were shown to act upon both B2 and B1 receptors, but it was demonstrated that those antagonists were substrates for carboxypeptidase N, and that the antagonist action on B1 receptors occurred only after cleavage of the antagonist to the

[des-Arg^9] analog. Newer, more potent, types of bradykinin antagonists contain residues at position 8 (such as Cpg, Oic) that block the degradative action of carboxypeptidase N; these antagonists have no action at B1 receptors. The present art of bradykinin antagonist peptides has not described any B1 antagonists that possess a carboxy-terminal arginine residue. Certain of the bradykinin antagonists described in this application that do contain the carboxy-terminal arginine residue have been found to possess high antagonist activity at both B1 and B2 receptors. This discovery runs counter to all principles generally accepted in the state of the art of bradykinin antagonists. Notwithstanding prior efforts, there remains a considerable need to provide improved B1 and B2 receptor antagonists. A main object of the present invention is to provide such receptor antagonists which include indaneglycine substituted bradykinin antagonists demonstrating B1 receptor and B2 receptor antagonist activity with high potency and broad specificity of antagonism.

SUMMARY OF THE INVENTION

The invention provides novel bradykinin antagonist peptides whereby, in the conventional bradykinin nomenclature, the amino acid residues at at least one of positions 5, 7, and 8, individually, pairwise or collectively, are replaced with indane-substituted glycine residues:

$$X\text{--}A^0\text{--}B^1\text{--}C^2\text{--}D^3\text{--}E^4\text{--}F^5\text{--}G^6\text{--}H^7\text{--}J^8\text{--}K^9\text{--}Z$$

Wherein

X is optionally absent but, if present, is an aromatic, aliphatic, aromatic-aliphatic, alicyclic,

Page missing at the time of publication

configuration, or a di- or poly-peptide containing amino acids of the D- or L- configuration, or a combination of these,

A is D-Arg or another basic or neutral aromatic, aliphatic, heterocyclic or alicyclic amino acid of the D- or L- configuration,

B is Arg or another basic or neutral aromatic, aliphatic, heterocyclic or alicyclic amino acid of the D- or L- configuration,

C is Pro, Hyp, or another basic or neutral aromatic, aliphatic, heterocyclic, or alicyclic amino acid of the D- or L- configuration,

D is Pro, Hyp, or another basic or neutral aromatic, aliphatic, heterocyclic, or alicyclic amino acid of the D- or L- configuration,

E is Gly, Niga, Nigb or another basic or neutral aromatic, aliphatic, heterocyclic, or alicyclic amino acid of the D- or L- configuration,

F is Igla, Iglb, Niga, Nigb, Phe, Thi, Cpg, Chg, or another aliphatic, aliphatic heterocyclic, or alicyclic amino acid of the D- or L- configuration,

G is Ser, Ser(SO), HBQ or another aromatic, aliphatic, heterocyclic, or alicyclic amino acid of the D- or L- configuration,

H is D-Igla, D-Iglb, Niga, Nigb, D-Tic, D-Cpg, D-Chg, or another aliphatic, aliphatic heterocyclic, or alicyclic amino acid of the D-configuration,

J is Igla, Iglb, Niga, Nigb, Tic, Nbn, Oic, Cpg, Chg, or another aromatic, aliphatic, aliphatic heterocyclic, or alicyclic amino acid of the D- or L- configuration,

K is Arg or another basic or neutral aromatic, aliphatic, heterocyclic or alicyclic amino acid of the D- or L- configuration, and

Z is the carboxy-terminal carboxyl group or a carboxy-terminal extension composed of an amino acid of the D- or L-configuration or a peptide composed of amino acids of the D- or L-configuration.

According to the above formula, X and Z may be alternatively described as being optionally absent in which case they may represent the terminal amino and carboxy groups, respectively.

The bradykinin antagonist peptides of the present invention may be illustrated by the following (alignment of the residues in a particular row does not imply nor limit to a given peptide sequence):

X--	A--	B--	C--	D--	E--	F--	G--	H--	J--	K--	Z
	0	1	2	3	4	5	6	7	8	9	
Aaa	DArg	Arg	Pro	Hyp	Gly	Igla	Ser	DIgla	Igla	Arg	
Aca	DLys	Lys	DMF	Pop	Iglb	Ser(SO)	DIglb	Iglb	Lys	DArg	
Atyl	Arg	DArg	NMF	Niga	Nigb	Niga	HBQ	Niga	Niga		
Dhq	Lys	DLys	HPIV	MPIV	Ala	Nigb	Cys	Nigb	Nigb		
Nba			Hyp	Pro	Gly	Leu	Gly	DLeu	Leu		
Tba			Azt	Azt		Chg	DIglb	DChg	Chg		
Cha			Dhp	Dhp		Ile		DIle	Ile		
Cpa			Inip	Inip		Val		DVal	Val		
Gun			Thz	Thz		Alg		DCpg	Cpg		
			Pop			Oic		DOic	Oic		
	Lys-Lys					Pop		DPop	Pop		
						Nle		DNle	Nle		
						DMF		DDMF			
								Iglb			

In a preferred embodiment, the bradykinin antagonist peptides of the present invention are represented as follows:

$$X\text{-Basic}^{0}\text{-Basic}^{1}\text{-Pro}^{2}\text{-Pro}^{3}\text{-Gly}^{4}\text{-Indanyl}^{5}\text{-Ser}^{6}\text{-DIndanyl}^{7}\text{-Indanyl}^{8}\text{-Basic}^{9}$$

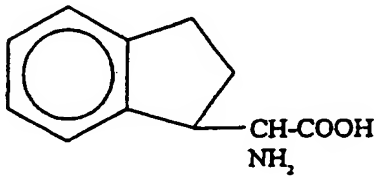
where "Indanyl" is a glycine residue substituted on either the α-carbon or the α-nitrogen by a 1-indanyl or a 2-indanyl moiety, and "Basic" indicates a basic amino acid residue, such as, for example, arginine or lysine.

A more preferred embodiment may be represented as follows:

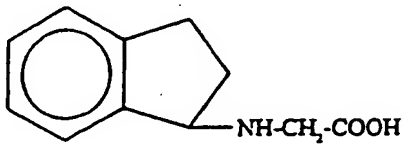
H-DArg^0-Arg^1-Pro^2-Hyp^3-Gly^4-Iglb^5-Ser^6-DIglb^7-Iglb^8-Arg^9.

Salts of the described bradykinin antagonist peptides include salts with HCl, TFA, HOAc, as well as other pharmaceutically acceptable salts.

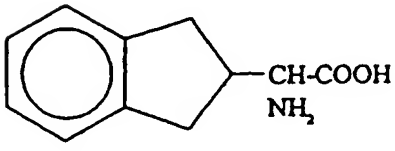
According to this invention, the indane substituent can be on either the α-carbon (residues abbreviated Igl) or the nitrogen (residues abbreviated Nig) of the glycine residue, and the indane residue can be attached to the glycine moiety at either position 1 (Igla or Niga) or position 2 (Iglb or Nigb) of the indane group.



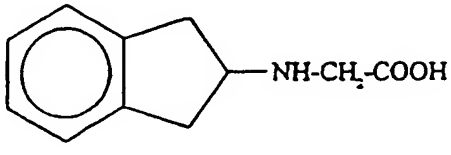
Igla



Niga



Iglb



Nigb

These indaneglycine substitutions in bradykinin antagonist peptides were made in addition to and in combination with other substitutions in bradykinin antagonist peptides known to those skilled in the art to yield effective antagonists of the biological actions of bradykinin. Several of the new bradykinin analogs containing indaneglycine were found to possess unexpectedly high potency in biological test systems. Also without precedent was the finding that certain of these novel bradykinin analogs were highly effective antagonists of bradykinin at both B1 and B2 receptors.

Often both B2 and B1 receptors are present on the same cells and when activated by either bradykinin or [des-Arg9]-bradykinin, the response produced is similar, e.g. vasodilatation or pain. B1 in addition to B2 receptors have been shown to mediate hypotension in animal models of sepsis and endotoxin shock (Regoli et al., *Eur. J. Pharmacol.*, 71: 105 (1981); Marceau et al., *Pharmacology*, 29:70 (1983); Nwator & Whalley, *Eur. J. Pharmacol.*, 160:125 (1989)); Siebeck et al., *Adv. Expy. Med. Biol.*, 247:389 (1989)); prostacyclin and nitric oxide release from endothelial cells (D'Orlean Juste et al., *Br. J. Pharmacol.*, 96:920 (1989); Wiemer & Wirth, *J. Pharm. Exp. Therap.*, 262:729 (1992)); edema (Neppl et al., *Acta Physiol. Scand.*, 142:141 (1991)); Manitone & Rodrigues, *Bt. J. Pharmacol.*, 99:516 (1990)); bone resorption (Ljunggren & Lerner, *Br. J. Pharmacol.*, 101:382 (1990)); stimulation of human gingival fibroblasts (Lerner & Moderer, *Inflammation*, 15:427 (1991)); release of inflammatory cytokines from macrophages (Burch et al., *Agents & Actions.*, 27:258 (1989)); hyperalgesia in acute and chronic inflammation (Dray & Perkins, *Trends Neurosci.*, 16:99 (1993)); Correa & Calixto, *Br. J.*

Pharmacol., 110:193 (1993), Cruwys et al., *Br. J. Pharmacol.*, 113:940 (1994); upregulation in antigen arthritis (Farmer et al., *Agents & Actions*, 34:191 (1991)); airways hyperreactivity and inflammatory cell influx into the lung (Farmer et al., *Br. J. Pharmacol.*, 107:653 1992)); contraction of the human colon, and non-pregnant and pregnant uterus (Couture et al., *Can, J. Physiol. Pharmacol.*, 59:957 (1981)); Abbas et al., *Brit J. Pharmacol.*, 112:433P (1993)); contraction of myofibroblasts (Appleton et al., Proc. of the Second International Symposium on cGRP-Peptides and Their Antagonists in Tissue Injury (Montreal, Aug.1994)); contraction of cerebral blood vessels (Whalley et al., *Naunyn-Schmiedebergs Arch. Pharmacol.*, 324:296 (1983)). (See also Hall *Pharmac. Ther.* 56: 131-190 (1992).

This invention also provides methods of treating and/or preventing disease conditions where the B1 and/or B2 receptor is implemented. Compounds with antagonist activity against both B2 and B1 receptors would be preferable than single receptor antagonist compounds in certain chronic diseases states. Based on the above description of B1 and B2 receptor involvement in disease, the compounds of the present invention are expected to be effective in treating SIRS (Systemic Inflammatory Response Syndromes)/sepsis, polytrauma, inflammatory bowel disease, acute and chronic pain, bone destruction in rheumatoid and osteo arthritis and periodontal disease, dysmenorrhea, premature labor, brain edema following focal injury, diffuse axonal injury, stroke, reperfusion injury and cerebral vasospasm after subarachnoid hemorrhage, allergic disorders including

asthma, adult respiratory distress syndrome, wound healing and scar formation.

As used herein, abbreviations of the natural amino acids are those accepted in the art (*Biochem. J.* 126: 773 (1972)), and unless prefixed with D are all of the L-configuration (except glycine and MPIV, which are not optically active).

Abbreviations used for unnatural amino acids in Bradykinin analogs are indicated below:

AC6	1-Aminocyclohexane-1-carboxylic acid
Aoc	2-Azabicylo (3.3.0) octane-3-carboxylic acid
Alg	Allylglycine
Azt	Azetine-2-carboxylic acid (norproline)
cAcp	2-Aminocyclopentane-1-carboxylic acid
CDF	*p*-Chloro-D-Phe
Chg	CyclohexylGly (α-Aminocyclohexaneacetic acid)
cLeu	1-Aminocyclopentane-1-carboxylic acid (cycloleucine)
Cpg	CyclopentylGly (α-Aminocyclopentaneacetic acid)
Dhp	3,4-Dehydro-Pro
DMF	2,4-Dimethylphenylalanine
DMTP	5,5-Dimethyl-4-thiaproline
Eac	6-Aminohexanoic acid (ϵ-aminocaproic acid)
FDF	*p*-Fluoro-DPhe
HBQ	N5-(4-hydroxybutyl)-glutamine
Hig	Hexahydroindanylglycine
Hyp	*trans*-4-Hydroxy-Pro
Ica	Indole 2-carboxylic acid
Igla	α-(1-indanyl)glycine
Iglb	α-(2-indanyl)glycine

Inip	Isonipecotic acid (piperidine-4-carboxylic acid)
Mag	α-Methallylglycine
MDY	O-Methyl-DTyr
MEF	2,3-Methano-E-phenylalanine
MPIV	2,4-Methanoproline (2-Azabicyclo-(2,1,1)-hexane-1-carboxylic acid)
Nal	β-2-Napthyl-Ala
Nia	N-(2-indanyl)-alanine
Niga	N-(1-indanyl)glycine
Nigb	N-(2-indanyl)glycine
Nle	Norleucine
NMF	N-Methylphenylalanine
Oic	Octahydroindole-2-carboxylic acid
OMT	O-Methyl-Tyr
Pal	β-3-Pyridyl-Ala
PCF	*p*-Chloro-Phe
Pip	Pipecolic acid ("homo-Pro")
Pop	*trans*-4-PropoxyPro
Ser(SO)	Serine-O-sulfate
Suc	Succinyl
Thi	β-2-Thienyl-Ala
Thz	Thiazolidine-4-carboxylic acid
Tic	1,2,3,4-Tetrahydroisoquinoline-3-carboxylic acid
TMF	2,4,6-Trimethylphenylalanine

Abbreviations used for derivatizing groups (as used for "X") are as follows:

Aaa-	1-Adamantaneacetyl-
Ac-	Acetyl-
Aca-	1-Adamantanecarboxyl-

Bpg-	N,N'-*bis*-Pentamethyleneguanidyl-
Bz-	Benzoyl-
Cha-	Cyclohexaneacetyl-
Cpa-	Cyclopentaneacetyl-
Dca-	2,2-Dicyclohexylacetyl-
Dcg-	N,N'-Dicyclohexylguanidyl-
Dhq-	2,3-Dehydroquinuclidine-3-carboxyl-
Dpa-	2,2-Diphenylacetyl-
Dpp-	3,3-Diphenylpropionyl-
Gun-	Guanidyl
Nba-	Norbornane-2-acetyl-
Nbc-	2-(*cis*-5-norbornene-*endo*-3-carboxyl)-
Nbi-	*cis*-5-norbornene-*endo*-2,3-dicarboximidyl-
Paa-	Phenylacetyl-
Pba-	4-Phenylbutyryl-
Ppa-	3-Phenylpropionyl-
Sin-	Sinapinyl-(3,5-dimethoxy-4-hydroxycinnamyl-)

The description of peptide synthesis methods uses several abbreviations for standard solvents, reagents and procedures, defined as follows:

BOP	Benzotriazolyloxy-tris-(dimethylamino)phosphonium hexafluorophosphate
BuOH	n-Butanol
DCC	Dicyclohexylcarbodiimide
DCM	Dichloromethane
DIC	Diisopropylcarbodiimide
DIEA	Diisopropylethyl amine
DMF	Dimethylformamide
HATU	O-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
HOAc	Acetic acid
MeOH	Methanol

OHMR	Hydroxymethylpolystyrene resin for peptide synthesis, 1% crosslinked.
TBTU	O-(benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate
TEA	Triethyl amine
TFA	Trifluoroacetic acid

The following abbreviations for blocking groups used in synthesis are:

Boc	*t*-Butyloxycarbonyl
Tos	*p*-Toluenesulfonyl
Bzl	Benzyl ether

The following abbreviations for standard techniques used are:

AAA	Amino acid analysis (Stewart & Young p. 108)
CCD	Countercurrent distribution (Stewart & Young p. 96)
ELEC	Paper electrophoresis (Stewart & Young p. 117)
HPLC	High performance liquid chromatography (Stewart & Young, p. 100)
Kaiser test	Ninhydrin test for completeness of coupling reactions (Stewart & Young, p. 105)
SPPS	Solid phase peptide synthesis
TLC	Thin-layer chromatography (Stewart & Young, p. 103)

The synthesis of peptides described herein, including preparation of appropriate amino acid derivatives, their activation and coupling to form

peptides and methods for purification of peptides and determination of their purity are included in the general body of knowledge of peptide chemistry, as generally described in Houben-Weyl "Methoden der Organischen Chemie" Vol. 16, parts I & II, (1974) for solution-phase synthesis, and in "Solid Phase Peptide Synthesis" by Stewart and Young (1984) for synthesis by the solid phase method. A chemist skilled in the art of peptide synthesis would be able to synthesize the described peptides by standard solution methods or by manual or automatic solid phase methods. The invention is described further by the following examples which are intended to be illustrative and instructive and in no way intended to be limiting.

EXAMPLES

EXAMPLE I - Peptide Synthesis - General Methods

Synthesis of the bradykinin antagonist peptides of the present invention by solid phase peptide synthesis (SPPS) may be carried out manually (see Stewart & Young) or by use of the Beckman Model 990, Biosearch Model 9500 or other automatic peptide synthesizers. SPPS involves use of certain standard procedures, defined as follows:

Procedure A: DCC coupling reaction: (Described in Stewart & Young, p 76ff). A 2.5-fold excess of Boc-amino acids over peptide-resin is used in the Model 990 synthesizer. Boc-amino acids are activated for coupling with an equimolar amount of DCC. The solvent may be DCM or mixtures of DCM and DMF. Completeness of coupling may be determined by use of the Kaiser reagent.

Procedure B: DIC coupling: In the Model 9500 synthesizer a 6-fold excess of Boc-amino acids over peptide-resin is used with an equimolar amount of DIC. The solvent is DCM:DMF (1:1). The resin is washed with the same solvent before and after coupling. Completeness of coupling is determined with the Kaiser reagent.

Procedure C: BOP, TBTU or HATU coupling reaction for hindered amino acids: A 3-fold excess of Boc-amino acid over peptide-resin is mixed with an equimolar amount of BOP, TBTU or HATU and 2 equivalents of DIEA in DMF. The peptide-resin is washed with DMF before and after the coupling reaction, and after coupling is then washed 2 times with methanol before continuing standard DCM washes. Completeness of coupling is checked by the Kaiser test. BOP, TBTU and HATU, in this order, show

increasing ability to cause successful coupling of sterically hindered amino acids.

Procedure D: TFA deprotection and neutralization: (Stewart & Young p. 76). The deprotection reagent is TFA:DCM (1:3), containing 1 mg/ml indole. It is used for 30 minutes, following a prewash. The neutralization reagent is 10% TEA in DCM, prepared fresh and used twice for one minute.

Procedure E: Terminal deprotection: (Described by Stewart & Young, p. 79). Deprotection with TFA:DCM is carried out as described in Procedure D. The peptide-resin is then washed three times with DCM and three times with MeOH and dried.

Procedure F: HF cleavage and deblocking: (Stewart & Young p. 85). A batch of 500mg (0.2 mmole) of peptide-resin is mixed with 1.0ml anisole and chilled in the reaction vessel to -78 C and 10ml of anhydrous HF is distilled into the vessel under vacuum. The mixture is stirred at 0 C for 45 min, and the HF is evaporated under vacuum. The peptide and resin mixture is washed three times with dry ether, and the peptide is extracted into glacial HOAc. The peptide solution is lyophilized.

Procedure G: PURIFICATION OF PEPTIDES: (Stewart & Young p. 96). The peptides may be purified by CCD for 100 transfers in the appropriate system, as

determined by preliminary *k* estimation. Examples of CCD systems are:

A: n-BuOH:1% TFA for average antagonist peptides

B: n-BuOH:ethyl acetate:1% TFA (1:1:2) for more hydrophobic antagonist peptides.

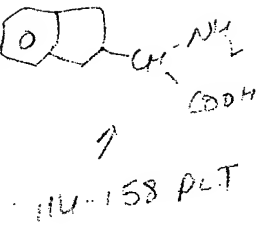
Procedure H: TLC: TLC may be carried out on silica gel plates with systems F (n-BuOH:HOAc:H_2O:pyridine =15:3:8:10) and I (n-BuOH:HOAc:H_2O=4:1:1). Chlorine-tolidine and Sakaguchi spray reagents may be used. (Stewart & Young, p. 120).

Procedure J: Paper electrophoresis (ELEC): ELEC may be done in buffers of Ph 2.8 and 5.0 as described in Stewart & Young. Chlorine-tolidine and Sakaguchi spray reagents may be used.

Procedure K: HPLC: Preparative HPLC may be carried out on large-pore reversed phase C4 or C8 silica columns in a gradient of 0.1% TFA in H_2O to 0.08% TFA in acetonitrile. Detection may be by UV at 214 or 235 nm. Analytical HPLC may be carried out in the same system and in a gradient of acetonitrile in 0.25*M* triethylammonium phosphate, pH 6.5.

Procedure L: MASS spectroscopy: Peptides may be checked for the correct molecular mass by fast atom bombardment (FAB) or laser desorption (MALDI) mass spectroscopy.

Procedure M: Amino acid analysis (AAA): Peptides may be hydrolyzed in 6*N* HCl and analyzed as described in Stewart & Young, pp 109-112, using a Beckman Model 6300 amino acid analyzer.



EXAMPLE II - Synthesis of α-(2-indane)-glycine (Iglb)

2-Bromoindane: To 2-indanol (Aldrich) (105 g, 0.78 mol) in pyridine (16 mL, 0.2 mol) and 340 mL of chloroform at -15 C was added PBr_3 (84 mL, 0.89 mol) over 45 min. The reaction mixture was stirred overnight at room temperature and extracted by addition of 450 mL of chloroform and 500 g of ice. The organic layer was washed twice with water, and dried over Na_2SO_4. The solvent was evaporated in vacuo, leaving a brown semi-solid. The product was distilled rapidly in vacuo and then fractionated to give 69g (45%) 2-bromoindane, bp 90-93 /3 mmHg; n_d^{23} = 1.5837.

Ethyl α-acetamido-α-cyano-2-indaneacetate: To sodium ethoxide (20.4 g, 0.3 mol), suspended in dry DMSO (250 mL) was added a solution of ethyl acetamidocyanoacetate (50 g, 0.294 mol) in 250 mL dry DMSO, with vigorous stirring. Then 2-bromoindane (65.0 g, 0.33 mol) was added dropwise during 40 min, with vigorous stirring. The brown solution was stirred overnight at room temperature and 4 h at 50 C. The

mixture was evaporated in vacuo and the residue was treated with 300 mL cold water and extracted twice with 250 mL of EtOAc. The combined extracts were dried ($MgSO_4$) and evaporated to give the crude product, 70.7 g brown solid. The first recrystallization from EtOH/H_2O gave 58.6 g yellowish solid, mp 153-157 . The second recrystallization from toluene gave 54.6 g (64.9%) white flakes, mp 159-161 C.

D,L-2-indaneglycine: A solution of 54.6 g (0.19 mol) ethyl α-acetamido-α-cyano-2-indaneacetate in 820 mL of 10% NaOH was refluxed for 20 h. The solution was cooled, decolorized with carbon, and the filtrate, in an ice bath, was adjusted to pH 6.5 with conc. HCl (about 150 mL), using a pH meter, and further chilled to complete precipitation of the product, which was filtered and washed with cold water, methanol and ether; 36.4 g. The solid was refluxed in 1200 mL 6N HCl for 10 h; the solution was decolorized with carbon and chilled to give a precipitate, which was collected and recrystallized from H_2O to give 22.6 g product. Additional product was obtained from the mother liquor to give 31.7 g (86.9%) of D,L-2-indaneglycine.

N-acetyl-D,L-2-indaneglycine: To a mixture of 6.83 g (0.03 mol) of D,L-2-indaneglycine and 54 mL H_2O was added 30 mL (0.06 mol) 2N NaOH. The solution was chilled and stirred while 1.4 mL (0.015 mol) acetic

anhydride was added. Seven successive additions of 2N NaOH (14 mL) and 1.4 mL of acetic anhydride were done over 30 min, and the solution was allowed to warm to room temperature, with continued stirring. Stirring was continued overnight. The solution was chilled and acidified to pH 3 with 6N H_2SO_4 (40 mL). After standing in the cold, the precipitate was collected and washed with a small amount of cold H_2O; 7.95 g, mp 199-202 . The product was recrystallized from acetone/petroleum ether (30-60) to give 7.8 g acetyl D,L-2-indaneglycine, mp 201-203 .

Resolution of D,L-2-indaneglycine: N-acetyl-D,L-2-indaneglycine (7.0 g, 0.03 mol) was suspended in 300 mL H_2O, and the pH was adjusted to 7.6 with 4N LiOH (9 mL). Water was added to a volume of 350 mL, the solution was thermostatted at 37 C, with stirring, and hog kidney acylase I (Sigma A-3010, 50 mg) was added. The pH was maintained at 7.6 by addition of LiOH; after precipitation of L-indaneglycine began, the pH rose and was brought back with 0.1 N HOAc. After 5 h an additional 30 mg acylase I was added, and after 24 h an additional 20 mg acylase was added, with continued pH control. Incubation, with stirring was for a total of 36 h. The solution was cooled to room temperature, and 250 mL cold water and 500 mL EtOAc were added. The mixture

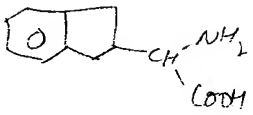
was carefully acidified to pH 0.75 with 6 N HCl (about 25 mL). The two layers were wet filtered through Celite and separated. The water phase was extracted twice with EtOAc (250 mL).

The aqueous solution was decolorized with carbon at 50 and evaporated under reduced pressure. The crystals were dissolved in 25 mL H_2O and 25 mL 6 N HCl and the cold solution was brought to pH 5.5 with conc. NH_4OH. The product was collected, washed with cold water, and dried; 2.8 g (97.6%) L-2-indaneglycine; mp 302-305 ; $[\alpha]_d^{25}$ = +35.4 (c 2, 2N HCl).

The ethyl acetate phase was washed with saturated NaCl solution, dried over $MgSO_4$ and evaporated in vacuo. The solid residue was recrystallized from EtOH/petroleum ether to give 3.1 g (88.6%) acetyl-D-2-indaneglycine, mp 210-212 ; $[\alpha]_d^{25}$ = -38.7 (c 2, EtOH).

Acetyl D-2-indaneglycine (2.92 g, 0.0125 mol) was refluxed in 125 mL of 6 N HCl for 8 H. The solution was evaporated under reduced pressure at 40 , and the residue was dissolved in 40 mL 6 N HCl and 150 mL H_2O. The solution was neutralized to pH 5.5 with conc. NH_4OH, and the white solid was collected, washed with cold water and dried in vacuo to yield 2.17 g (90.8%) of D-2-indaneglycine; mp 302-305 ; $[\alpha]_d^{23}$ = -34.6 (c 2, 2N HCl).

Both the D- and L-isomers were converted to the N-Boc derivative by the standard procedure described in Example III. The mp and rotation of the compound is: mp 86-89°C (dec); $[\alpha]_D^{22}$=+16.9 (c2, EtOH).

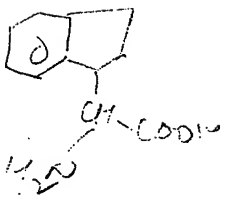


EXAMPLE III - Synthesis of N-Boc-N-(2-indanyl) glycine(Boc-Nigb)

Synthesis of N-(2-indanyl)-glycine (Nigb): Glycine methyl ester (3.49g, 0.025 mole) and 2-indanone (4.96g, 0.0375 mole) were dissolved in EtOH and then $NaCNBH_3$ (4.71g, 0.075 mole) was added portionwise during about 30 min. The mixture was stirred at room temperature for 24 h. The EtOH was removed under reduced pressure and the residue was treated with water. The product was extracted by several extractions with EtOAc. The organic phase was washed with saturated aqueous $NaHCO_3$, dried over Na_2SO_4 and evaporated. The product was purified by chromatography over silica gel (EtOAc and EtOAc/MeOH = 9:1) to give the crude ester as a colorless oil (2.46 g). The ester (2.43 g in 30mL MeOH) was saponified with 15 mL 1N NaOH at room temperature for 5 h. The solution was concentrated and the residue was taken up in dioxane-water.

Synthesis of the Boc-derivative: The solution was cooled to 0 and treated with Boc anhydride (3.27 g,

0.015 mole) portionwise. The ice bath was removed, and the mixture was stirred overnight at room temperature, with adjustment of pH to 9.0 with NaOH solution as needed. The solution was evaporated to dryness under reduced pressure, the residue was taken up in EtOAc/water (70/30 mL), and the solution was treated with saturated aqueous citric acid solution to pH 2.5. The phases were separated and the water was extracted twice with EtOAc. The organic phase was washed with water, saturated NaCl solution, dried over Na_2SO_4 and evaporated under reduced pressure. The crude product was recrystallized from EtOAc/hexanes; 1.85 g (25.4%), mp 130-131 C.



EXAMPLE IV - Synthesis and Derivatization of α-(1-Indane)Glycine

The D- and L-isomers of α-(1-indane)glycine were synthesized as described by Josien et al., *J. Med. Chem.* 37: 1586-1601 (1994). The synthetic procedures gave the optically pure D- and L-amino acids.

The amino acids were converted to the N-Boc derivatives by the standard procedure described in Example III; m.p. 109-111 .

EXAMPLE V - Synthesis and Derivatization of N-(1-Indanyl)Glycine

The amino acid was synthesized as described by Miyake, et al., *Takeda Kenkyushoho* 44: 171-185 (1985). [*Chem. Abstr.* 106: 156830 (1987)]. N-(1-indanyl)-benzylamine was prepared by reductive amination of 1-indanone with benzylamine, and alkylation of the amine by ethyl bromoacetate. The benzyl group was cleaved by hydrogenolysis over palladium. Saponification of the ethyl ester gave the amino acid, which was converted to the Boc derivative by the standard procedure described in Example III.

Alternatively, 1-indanone was reductively aminated with glycine methyl ester, followed by saponification of the ester.

The following examples are illustrative of compounds of this invention and are not limitative. All percentages and ratios are by weight when solids are involved and by volume when only liquids are involved.

EXAMPLE VI - Synthesis of Compound 1 - DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Oic-Arg

The Model 990 synthesizer vessel was loaded with 1.66g of Boc-Arg(Tos)-OHMR (0.24 mmol/g substitution;

0.4mmol total). After deprotection and neutralization by Procedure D, Boc-L-Oic was coupled by Procedure C, using BOP as coupling agent. Coupling was checked for completeness with the Kaiser test, and may be repeated if necessary. In the same manner, Boc-D-Iglb, Boc-Ser(Bzl), Boc-Iglb, Boc-Gly, Boc-Hyp, Boc-Pro, Boc-Arg(Tos) and Boc-D-Arg(Tos) were coupled, using procedure C with BOP reagent. After deprotection by Procedure E, the peptide-resin was divided into 4 parts. The peptide was cleaved from one part of the resin and deblocked by Procedure F. The peptide was purified by CCD using Procedure G and System A. The purified peptide was checked for purity by TLC (Procedure H), ELEC (Procedure J) and HPLC (Procedure K), and characterized by AAA (Procedure M) and MASS (Procedure L). The other parts of the peptide-resin were used for synthesis of the derivatives in which acyl groups were attached to the amino-terminus of the peptide on the resin.

EXAMPLE VII - Synthesis of Compound 2 - Aca-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Oic-Arg

Acylation of the peptide while on the resin: Using one part of the peptide-resin prepared in Example VI, Adamantanecarboxylic acid was coupled to

the peptide using Procedure C with TBTU reagent to activate the Aca. The acylated peptide was cleaved from the resin, purified and characterized by the same procedures described in Example VI.

EXAMPLE VIII - Synthesis of Compound 3 - Dhq-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Oic-Arg

Acylation of the pure peptide in solution: A sample of 25mg of the pure peptide of Example VI was dissolved in 0.5ml DMF and two equivalents of DIEA were added. 2,3-Dehydroquinuclidine-3-carboxylic acid (1.5 equivalents) was dissolved in 0.5ml DMF and activated by addition of BOP reagent (1.5 equivalents) and DIEA (3 equivalents). After activation for 15min at room temperature, the twoacylating mixture was added to the peptide solution. The solution was stirred overnight, and then evaporated in high vacuum. The acylated peptide was purified by HPLC (Procedure K) and characterized using Procedures H, J, L and M.

EXAMPLE IX - Solid phase synthesis of Compound 4 - DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-MPIV-Arg

Use of HATU reagent for coupling a sterically highly hindered Boc-amino acid to a peptide-resin: The

synthesis of this peptide was carried out as described in Example VI, except that the Boc-MPIV failed to couple to the Arg-resin when activated by BOP. Therefore Boc-MPIV was activated with HATU, when coupling proceeded well. The remainder of the synthesis was carried out with BOP reagent for activation. Cleavage, purification, and characterization of this peptide was done as in Example VI.

EXAMPLE X -

Using the same general procedures, the following peptides were synthesized, purified and characterized:

5. Arg-Pro-Hyp-Gly-Thi-Ser-DIglb-Oic-Arg
6. DArg-Arg-Pro-Hyp-Gly-Thi-Ser-Iglb-Oic-Arg
7. DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIglb-Oic-Arg
8. DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DCpg-Iglb-Arg
9. DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DCpg-DIglb-Arg
10. DArg-Arg-Pro-Hyp-Gly-Cpg-Ser-DCpg-Iglb-Arg
11. DArg-Arg-Pro-Hyp-Gly-Cpg-Ser-DCpg-DIglb-Arg
12. DArg-Arg-Pro-Hyp-Gly-Cpg-Ser-DTic-Iglb-Arg
13. DArg-Arg-Pro-Hyp-Gly-Cpg-Ser-DTic-DIglb-Arg
14. DArg-Arg-Pro-Hyp-Gly-Phe-Ser-Iglb-Oic-Arg
15. DArg-Arg-Pro-Hyp-Gly-Phe-Ser-DIglb-Oic-Arg
16. DArg-Arg-Pro-Hyp-Gly-Thi-Ser-Nigb-Oic-Arg
17. DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DTic-Oic-Arg
18. DArg-Arg-Pro-Hyp-Gly-DIglb-Ser-DTic-Oic-Arg

19. DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIgl-Iglb-Arg

20. DArg-Arg-Pro-Hyp-Gly-Thi-Ser-Cpg-Nigb-Arg

21. DArg-Arg-Pro-Hyp-Gly-Thi-Ser-Iglb-Tic-Arg

22. DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIglb-Tic-Arg

23. DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DTic-Nigb-Arg

24. DArg-Arg-Pro-Hyp-Gly-Cpg-Ser-DIglb-Oic-Arg

25. DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DTic-Oic-Arg

26. DArg-Arg-Pro-Hyp-Gly-DMF-Ser-DIglb-Oic-Arg

27. DArg-Arg-Pro-Hyp-Gly-DDMF-Ser-DIglb-Oic-Arg

28. DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIgl-Nigb-Arg

29. DArg-Arg-Pro-Hyp-Gly-Cpg-Ser-DTic-Nigb-Arg

30. DArg-Arg-Pro-Hyp-Gly-DIglb-Ser-DIglb-Oic-Arg

31. DArg-Arg-Pro-Hyp-Gly-DThi-Ser-DIglb-Oic-Arg

32. DArg-Arg-Pro-Hyp-Gly-Nigb-Ser-DIglb-Oic-Arg

33. DArg-Arg-Pro-Hyp-Nigb-Thi-Ser-DIglb-Oic-Arg

34. DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Cpg-Arg

35. DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIglb-Leu-Arg

36. DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIglb-Thi-Arg

37. DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIglb-AC6-Arg

38. DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIglb-Ile-Arg

39. DArg-Arg-Pro-Hyp-Gly-Thi-HBQ-DIglb-Oic-Arg

40. DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIglb-cLeu-Arg

41. DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIglb-Dic-Arg

42. DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIglb-Nbn-Arg

43. DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DTic-Nigb-Arg

44. DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Iglb-Arg

45. DArg-Arg-NMF-Hyp-Gly-Iglb-Ser-DIglb-Oic-Arg

46. DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DCpg-Cpg-Arg

47. Eac-DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIglb-Oic-Arg
    |
    Suc-DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIglb-Oic-Arg

48. Aca-DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DTic-Nigb-Arg

49. Aca-DArg-Arg-Pro-Hyp-Gly-Thi-Ser-Nigb-Oic-Arg

50. Dhq-DArg-Arg-Pro-Hyp-Gly-Cpg-Ser-DTic-Iglb-Arg
51. Dhq-DArg-Arg-Pro-Hyp-Gly-Cpg-Ser-DTic-DIglb-Arg
52. Aca-DArg-Arg-Pro-Hyp-Gly-Phe-Ser-Iglb-Oic-Arg
53. Aca-DArg-Arg-Pro-Hyp-Gly-Phe-Ser-DIglb-Oic-Arg
54. Aca-DArg-Arg-Pro-Hyp-Gly-Thi-Ser-Iglb-Oic-Arg
55. Aca-DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIglb-Oic-Arg
56. Aaa-DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIglb-Oic-Arg
57. DCha-DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIglb-Oic-Arg
58. Dcg-DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIglb-Oic-Arg
59. Dhq-DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIglb-Oic-Arg
60. Sin-DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIglb-Oic-Arg
61. Aca-DArg-Arg-Pro-Hyp-Gly-Thi-Ser(SO)-DIglb-Oic-Arg
62. Aca-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DTic-Oic-Arg
63. Aaa-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Oic-Arg
64. Dca-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Oic-Arg
65. Dpa-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Oic-Arg
66. Dpp-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Oic-Arg
67. Nbc-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Oic-Arg
68. Nbi-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Oic-Arg
69. Paa-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Oic-Arg
70. Pba-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Oic-Arg
71. Ppa-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Oic-Arg
72. Sin-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Oic-Arg
73. Aaa-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Iglb-Arg
74. Aca-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Iglb-Arg
75. Dca-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Iglb-Arg
76. Dhq-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Iglb-Arg
77. Dpa-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Iglb-Arg
78. Dpp-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Iglb-Arg
79. Nbc-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Iglb-Arg
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81. Paa-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Iglb-Arg
82. Pba-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Iglb-Arg
83. Ppa-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Iglb-Arg
84. Sin-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Iglb-Arg
85. DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIgla-Niga-Arg
86. DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIgla-Igla-Arg
87. DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIgla-Tic-Arg
88. DArg-Arg-Pro-Hyp-Gly-Igla-Ser-DTic-Niga-Arg
89. DArg-Arg-Pro-Hyp-Gly-Cpg-Ser-DIgla-Oic-Arg
90. DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIgla-Oic-Arg
91. DArg-Arg-Pro-Hyp-Gly-Igla-Ser-DTic-Oic-Arg

92. DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIgla-Niga-Arg
93. DArg-Arg-Pro-Hyp-Gly-Nigb-Ser-DIgla-Oic-Arg
94. DArg-Arg-Pro-Hyp-Gly-Igla-Ser-DIglb-Cpg-Arg
95. DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Niga-Arg
96. DArg-Arg-Pro-Hyp-Gly-Igla-Ser-DIglb-Thi-Arg
97. DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIgla-Ile-Arg
98. DArg-Arg-Pro-Hyp-Gly-Igla-Ser-DIgla-Oic-Arg
99. Aaa-DArg-Arg-Pro-Hyp-Gly-Igla-Ser-DIgla-Oic-Arg
100. Aca-DArg-Arg-Pro-Hyp-Gly-Igla-Ser-DIgla-Oic-Arg
101. Dhq-DArg-Arg-Pro-Hyp-Gly-Igla-Ser-DIgla-Oic-Arg
102. Dca-DArg-Arg-Pro-Hyp-Gly-Igla-Ser-DIgla-Oic-Arg
103. Dpa-DArg-Arg-Pro-Hyp-Gly-Igla-Ser-DIgla-Oic-Arg
104. Dpp-DArg-Arg-Pro-Hyp-Gly-Igla-Ser-DIgla-Oic-Arg
105. Nbc-DArg-Arg-Pro-Hyp-Gly-Igla-Ser-DIgla-Oic-Arg
106. Nbi-DArg-Arg-Pro-Hyp-Gly-Igla-Ser-DIgla-Oic-Arg
107. Paa-DArg-Arg-Pro-Hyp-Gly-Igla-Ser-DIgla-Oic-Arg
108. Pba-DArg-Arg-Pro-Hyp-Gly-Igla-Ser-DIgla-Oic-Arg
109. Ppa-DArg-Arg-Pro-Hyp-Gly-Igla-Ser-DIgla-Oic-Arg
110. Sin-DArg-Arg-Pro-Hyp-Gly-Igla-Ser-DIgla-Oic-Arg
111. DArg-Arg-Pro-Hyp-Gly-Igla-Ser-DIgla-Igla-Arg
112. Aaa-DArg-Arg-Pro-Hyp-Gly-Igla-Ser-DIgla-Igla-Arg
113. Aca-DArg-Arg-Pro-Hyp-Gly-Igla-Ser-DIgla-Igla-Arg
114. Dca-DArg-Arg-Pro-Hyp-Gly-Igla-Ser-DIgla-Igla-Arg
115. Dhq-DArg-Arg-Pro-Hyp-Gly-Igla-Ser-DIgla-Igla-Arg
116. Dpa-DArg-Arg-Pro-Hyp-Gly-Igla-Ser-DIgla-Igla-Arg
117. Dpp-DArg-Arg-Pro-Hyp-Gly-Igla-Ser-DIgla-Igla-Arg
118. Nbc-DArg-Arg-Pro-Hyp-Gly-Igla-Ser-DIgla-Igla-Arg
119. Nbi-DArg-Arg-Pro-Hyp-Gly-Igla-Ser-DIgla-Igla-Arg
120. Paa-DArg-Arg-Pro-Hyp-Gly-Igla-Ser-DIgla-Igla-Arg
121. Pba-DArg-Arg-Pro-Hyp-Gly-Igla-Ser-DIgla-Igla-Arg
122. Ppa-DArg-Arg-Pro-Hyp-Gly-Igla-Ser-DIgla-Igla-Arg
123. Sin-DArg-Arg-Pro-Hyp-Gly-Igla-Ser-DIgla-Igla-Arg
124. Aca-DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DHigb-Oic-Arg
125. DArg-Arg-Pro-Hyp-Gly-Thi-Ser-Higb-Oic-Arg
126. DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DHigb-Oic-Arg
127. Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Oic-Arg
128. DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Higb-Arg
129. DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-Iglb-Higb-Arg
130. DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIglb-Higb-Arg
131. DArg-Arg-Pro-Hyp-Gly-Thi-Ser-Iglb-Higb-Arg
132. DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIglb-Hyp-Arg
133. Aaa-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Oic-Arg

134. Aca-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Oic-Arg
135. Dhq-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Oic-Arg
136. Sin-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Oic-Arg
137. Aaa-Arg-Pro-Hyp-Gly-Thi- Ser-DIglb-Oic-Arg
138. Aca-Arg-Pro-Hyp-Gly-Thi-Ser-DIglb-Oic-Arg
139. Dhq-Arg-Pro-Hyp-Gly-Thi-Ser-DIglb-Oic-Arg
140. Sin-Arg-Pro-Hyp-Gly-Thi-Ser-DIglb-Oic-Arg
141. Gun-DArg-Arg-Pro-Hyp-Gly-Igla-Ser-DIgla-Igla-Arg
142. Gun-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Oic-Arg
143. Gun-DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIglb-Oic-Arg
144. Gun-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Aoc-Arg
145. DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Aoc-Arg
146. DArg-Arg-Pro-Hyp-Gly-Phe-Ser-DIglb-Aoc-Arg
147. DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIglb-Aoc-Arg
148. DArg-Arg-Pro-Hyp-Gly-Cpg-Ser-DIglb-Aoc-Arg
149. DArg-Arg-Pro-Pro-Gly-Iglb-Ser-DIglb-Iglb-Arg
150. DArg-Arg-Pro-Pro-Gly-Iglb-Ser-DIglb-Oic-Arg
151. DArg-Arg-Pro-Pro-Gly-Iglb-Ser-DIglb-Aoc-Arg
152. Aaa-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Aoc-Arg
153. Aca-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Aoc-Arg
154. Dca-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Aoc-Arg
155. Dhq-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Aoc-Arg
156. Dpa-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Aoc-Arg
157. Dpp-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Aoc-Arg
158. Nbc-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Aoc-Arg
159. Nbi-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Aoc-Arg
160. Paa-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Aoc-Arg
161. Pba-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Aoc-Arg
162. Ppa-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Aoc-Arg
163. Sin-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Aoc-Arg
164. Gun-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Oic-Arg
165. Gun-DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIglb-Oic-Arg
166. DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Ica-Arg
167. DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIglb-Pip-Arg
168. DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DNal-Oic-Arg
169. DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIglb-DMTP-Arg
170. DArg-Arg-Pro-Hyp-Gly-Iglb-Lys-DIglb-Oic-Arg
171. DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Chg-Arg
172. Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Oic-Arg
173. DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIglb-Tyr-Arg
174. DArg-Arg-Pro-Hyp-Gly-Iglb-Thr-DIglb-Oic-Arg
175. DArg-Arg-Pro-Hyp-Gly-Thi-Glu-DIglb-Oic-Arg

176. DArg-Arg-Pro-Hyp-Gly-His-Ser-DIglb-Oic-Arg
177. Arg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Oic-Arg
178. DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIglb-Trp-Arg
179. DArg-Arg-Pro-Hyp-Gly-TMF-Ser-DIglb-Oic-Arg
180. DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-MPIV-Arg
181. DArg-Arg-Pro-Hyp-Gly-DMF-Ser-DIglb-Oic-Arg
182. DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIglb-DMF-Arg
183. DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DTic-Oic-Arg
184. DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DAlg-Igl-Arg
185. DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DTic-Nia-Arg
186. DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIglb-Nia-Arg
187. DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-MEF-Arg
188. DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIglb-MEF-Arg
189. DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIglb-TMF-Arg
190. DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-TMF-Arg
191. DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Alg-Arg
192. DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Mag-Arg
193. DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DMag-Igl-Arg
194. DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DMag-Oic-Arg
195. DArg-Arg-Pro-Pro-Gly-Iglb-Ser-DMag-Aoc-Arg
196. Gun-DArg-Arg-Pro-Hyp-Gly-cAcp-Ser-DIglb-Oic-Arg
197. Gun-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Ica-Arg
198. Gun-DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIglb-Pip-Arg
199. Gun-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DNal-Oic-Arg
200. Gun-DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIglb-DMTP-Arg
201. Gun-DArg-Arg-Pro-Hyp-Gly-Iglb-Lys-DIglb-Oic-Arg
202. Gun-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Chg-Arg
203. Gun-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Oic-Arg
204. Gun-DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIglb-Tyr-Arg
205. Gun-DArg-Arg-Pro-Hyp-Gly-Iglb-Thr-DIglb-Oic-Arg
206. Gun-DArg-Arg-Pro-Hyp-Gly-Thi-Glu-DIglb-Oic-Arg
207. Gun-DArg-Arg-Pro-Hyp-Gly-His-Ser-DIglb-Oic-Arg
208. Gun-Arg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Oic-Arg
209. Gun-DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIglb-Trp-Arg
210. Gun-DArg-Arg-Pro-Hyp-Gly-TMF-Ser-DIglb-Oic-Arg
211. Gun-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-MPIV-Arg
212. Gun-DArg-Arg-Pro-Hyp-Gly-DMF-Ser-DIglb-Oic-Arg
213. Gun-DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIglb-DMF-Arg
214. Gun-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DTic-Oic-Arg
215. Gun-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DAlg-Igl-Arg
216. Gun-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DTic-Nia-Arg
217. Gun-DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIglb-Nia-Arg

218. Gun-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-MEF-Arg
219. Gun-DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIglb-MEF-Arg
220. Gun-DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIglb-TMF-Arg
221. Gun-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-TMF-Arg
222. Gun-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Alg-Arg
223. Gun-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Mag-Arg
224. Gun-DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DMag-Igl-Arg
225. Gun-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DMag-Oic-Arg
226. Gun-DArg-Arg-Pro-Pro-Gly-Iglb-Ser-DMag-Aoc-Arg
227. Dcg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Oic-Arg
228. Dcg-DArg-Arg-Pro-Hyp-Gly-cAcp-Ser-DIglb-Oic-Arg
229. Dcg-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Ica-Arg
230. Dcg-DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIglb-Pip-Arg
231. Dcg-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DNal-Oic-Arg
232. Dcg-DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIglb-DMTP-Arg
233. Dcg-DArg-Arg-Pro-Hyp-Gly-Iglb-Lys-DIglb-Oic-Arg
234. Dcg-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Chg-Arg
235. Dcg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Oic-Arg
236. Dcg-DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIglb-Tyr-Arg
237. Dcg-DArg-Arg-Pro-Hyp-Gly-Iglb-Thr-DIglb-Oic-Arg
238. Dcg-DArg-Arg-Pro-Hyp-Gly-Thi-Glu-DIglb-Oic-Arg
239. Dcg-DArg-Arg-Pro-Hyp-Gly-His-Ser-DIglb-Oic-Arg
240. Dcg-Arg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Oic-Arg
241. Dcg-DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIglb-Trp-Arg
242. Dcg-DArg-Arg-Pro-Hyp-Gly-TMF-Ser-DIglb-Oic-Arg
243. Dcg-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-MPIV-Arg
244. Dcg-DArg-Arg-Pro-Hyp-Gly-DMF-Ser-DIglb-Oic-Arg
245. Dcg-DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIglb-DMF-Arg
246. Dcg-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DTic-Oic-Arg
247. Dcg-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DAlg-Igl-Arg
248. Dcg-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DTic-Nia-Arg
249. Dcg-DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIglb-Nia-Arg
250. Dcg-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-MEF-Arg
251. Dcg-DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIglb-MEF-Arg
252. Dcg-DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIglb-TMF-Arg
253. Dcg-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-TMF-Arg
254. Dcg-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Alg-Arg
255. Dcg-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Mag-Arg
256. Dcg-DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DMag-Igl-Arg
257. Dcg-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DMag-Oic-Arg
258. Dcg-DArg-Arg-Pro-Pro-Gly-Iglb-Ser-DMag-Aoc-Arg
259. Dcg-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Oic-Arg

260. DArg-Arg-Pro-Hyp-Gly-cAcp-Ser-DIglb-Oic-Arg
261. Bpg-DArg-Arg-Pro-Hyp-Gly-cAcp-Ser-DIglb-Oic-Arg
262. Bpg-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Ica-Arg
263. Bpg-DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIglb-Pip-Arg
264. Bpg-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DNal-Oic-Arg
265. Bpg-DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIglb-DMTP-Arg
266. Bpg-DArg-Arg-Pro-Hyp-Gly-Iglb-Lys-DIglb-Oic-Arg
267. Bpg-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Chg-Arg
268. Bpg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Oic-Arg
269. Bpg-DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIglb-Tyr-Arg
270. Bpg-DArg-Arg-Pro-Hyp-Gly-Iglb-Thr-DIglb-Oic-Arg
271. Bpg-DArg-Arg-Pro-Hyp-Gly-Thi-Glu-DIglb-Oic-Arg
272. Bpg-DArg-Arg-Pro-Hyp-Gly-His-Ser-DIglb-Oic-Arg
273. Bpg-Arg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Oic-Arg
274. Bpg-DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIglb-Trp-Arg
275. Bpg-DArg-Arg-Pro-Hyp-Gly-TMF-Ser-DIglb-Oic-Arg
276. Bpg-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-MPIV-Arg
277. Bpg-DArg-Arg-Pro-Hyp-Gly-DMF-Ser-DIglb-Oic-Arg
278. Bpg-DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIglb-DMF-Arg
279. Bpg-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DTic-Oic-Arg
280. Bpg-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DAlg-Igl-Arg
281. Bpg-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DTic-Nia-Arg
282. Bpg-DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIglb-Nia-Arg
283. Bpg-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-MEF-Arg
284. Bpg-DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIglb-MEF-Arg
285. Bpg-DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIglb-TMF-Arg
286. Bpg-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-TMF-Arg
287. Bpg-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Alg-Arg
288. Bpg-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Mag-Arg
289. Bpg-DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DMag-Igl-Arg
290. Bpg-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DMag-Oic-Arg
281. Bpg-DArg-Arg-Pro-Pro-Gly-Iglb-Ser-DMag-Aoc-Arg
292. Bpg-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Oic-Arg
293. Bpg-DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIglb-Oic-Arg

(Compound 47 represents a dimeric compound).

EXAMPLE XI - Biological Activity

The bradykinin antagonists were assayed on isolated rat uterus in natural or induced estrus and on guinea pig ileum, according to the commonly

accepted assay methods for bradykinin and related kinins as described by Trautschold (Handbook of Experimental Pharmacology, Vol. 25, Springer-Verlag, pp 53-55, (1969)) for inhibition of the myotropic activity of bradykinin. The inhibition potencies were determined according to the commonly accepted manner, as described by Schild for antagonists of biologically active compounds (*Brit. J. Pharmacol.* 2: 189 (1947)) and expressed as pA_2 values. In the assays, a dose-response curve was determined for the reference substance bradykinin. The dose of bradykinin which produces a half-maximal contraction of the tissue is the ED_{50} dose. An amount of bradykinin equivalent to twice the ED_{50} dose was administered to the tissue 30 seconds after the start of incubation of the tissue with a dose of antagonist. Doses of antagonist were increased in this protocol until the dose of antagonist was found that caused the tissue response to a double ED_{50} dose of bradykinin in the presence of antagonist to equal the response of an ED_{50} dose of bradykinin without antagonist. The pA_2 value, therefore, represents the negative logarithm of the molar concentration of antagonist necessary to reduce the response to a double ED_{50} dose of bradykinin to that of an ED_{50} dose without antagonist. A change of one unit of pA_2 value represents an order of magnitude change in potency. For comparison, the negative logarithm of the dose of bradykinin that causes half-maximal contraction of the tissues, commonly known as the pD_2 value, is 7.9 on the rat uterus and 7.4 on the guinea pig ileum.

The *in vivo* effects of bradykinin antagonists on blood pressure in the anesthetized rat were determined according to the assay described by Roblero, Ryan and

Stewart (*Res. Commun. Pathol. Pharmacol.* 6: 207 (1973)). The antagonists produce inhibition of the hypotensive action of bradykinin when administered as a bolus admixture of bradykinin plus antagonist or when administered as an infusion. Potencies for the antagonists in this assay are not reported precisely, but rather are indicated approximately.

On isolated smooth muscles, agonist potency is given as percent of BK potency; antagonist potency is given as pA_2, and is underlined. In blood pressure assays, the number is the dose of peptide in micrograms that causes 50% reduction in the effect of BK intraarterially administered as a bolus mixture in a 500g male rat; Ag indicates unquantitated agonist activity; Ant indicates unquantitated antagonist activity.

TABLE I: Biological Activities of Typical Peptide Examples

Compound Number	Uterus Activity	Ileum Activity	Blood Pressure
1.	8.5	7.9	0.5
2.	7.5	7.8	5
3.	7.4	7.6	5
5.		7.7	
7.	7.8	7.4	5
9.	7.4	7.2	5
12.	10.5%	5.1	0
13.	8.6%	6.0	0
14.	6.5	5.1	0
15.	7.9	7.6	0.05
16.	6.8	7.0	0.5
17.	Ant	Ant	5
18.			5
19.	9.1%	6.7	Ag
22.	7.0	6.8	0.5
23.	8.3	7.2	0.5
24.		8.1	5
25.	0.7%	7.8	0.5
26.	7.3	6.6	5
27.	8.0	6.9	0.5
28.	48.6%	6.5	0.05

29.	8.1	7.6	5
30.	8.0	7.8	0.5
31.	8.2	6.0	5
32.		5.3	
33.	5.0	5.1	
34.	7.8	6.8	0.05
35.	3.3%	6.8	
36.	3.0%	5.4	50
37.	7.0	6.0	
38.		6.8	0.5
39.	7.1		5
40.			5
41.			50
43.			0.5
44.			50
45.		6.5	0.5
48.	6.0		
49.	7.1	6.5	50
50.		5.4	5
51.	0.3%	5.8	50
52.	7.2	5.9	0
53.	8.2	7.4	0.5
54.	5.8		
55.	9.2	8.4	0.5
56.	7.7	7.8	5
58.	3.0%	5.4	
59.	8.1	7.2	0.5
60.	8.3	7.3	0.05
61.		7.5	0.5
124.		7.7	0
125.	9.4%	6.6	Ag

EXAMPLE XII - Human Ileum

Human ilea were obtained from a human tissue bank (International Institute for the Advancement of Medicine, Exton, PA). Sections 25 mm x 5 mm were placed under 2gm isometric resting tension in 4 ml tissue baths containing Krebs-Henseleit solution and bubbled with 95% O_2 and 5% CO_2. Concentration-effect curves were constructed to BK in the absence and presence of different doses of antagonist. The antagonist potency (pA_2, i.e. the negative log of the concentration of the antagonist which produced a

2-fold shift in the concentration-effect curve to bradykinin) was calculated using the method of Schild (1947).

Compound	Human Ileum B2 receptor	n
1	8.75 ± 0.08	7
7	7.79 ± 0.18	13

EXAMPLE XIII - B1 Receptor Bioassay

Male New Zealand white rabbits, weight range 2-3 kg, were killed by an overdose of pentobarbital i.v. The thorax was opened and the thoracic aorta carefully removed. Spiral strips, approximately 25 x 3-4 mm were prepared and placed in 4 ml tissue baths containing Krebs solution at 37°C and bubbled with 95% O_2 and 5% CO_2. The tissues were placed under 1 gm isometric tension and allowed to equilibrate for 1H. At time 1h, a concentration-effect curve was constructed to the selective B1 agonist des-Arg^9-BK. This was repeated at time 3 hours. At time 5h des-Arg^9-BK was added to the tissue bath at a final concentration of 10^{-7}M. This produced a sustained contraction that could be maintained for approximately 45 min. Once the contraction was sustained, the compound to be assayed was applied to the tissue bath in a cumulative manner. The concentration of the compound which produced a 50% reduction of the sustained contraction was calculated. The negative logarithm of this concentration was calculated and expressed as the IC_{50}. All compounds were compared to the standard B1 antagonist des-Arg^{10}-Leu^9-kallidin which had a pIC_{50} of 7.9.

Compound	Rabbit Aorta p IC50 B1 receptors	n
1	6.60 ± 0.03	4
2	5.75 ± 0.23	5
3	5.93 ± 0.17	5
5	5.81 ± 0.19	5
7	6.85 ± 0.21	6
11	6.4 ± 0.03	3
15	6.79 ± 0.27	3
22	5.75 ± 0.03	8
23	<5	5
24	6.37 ± 0.08	8
25	5.32 ± 0.06	6
27	<5	3
28	<5	4
30	<5	3
31	<5	3
45	5.54 ± 0.08	6
47	5.35 ± 0.18	4
53	5.9 ± 0.10	2
55	6.57 ± 0.21	6
56	6.16 ± 0.29	2
60	5.68 ± 0.13	6
61	5.38 ± 0.08	4

EXAMPLE XIV - Human B2 Binding

Human B2 receptors were cloned and expressed in Chinese hamster ovary cells. RNA was isolated from human lung fibroblasts (CCD-16 LU obtained from the ATCC) using the method of Chirgwin et al *(Biochemistry* 18:5294 (1979)). The RNA was transcribed into cDNA using MMLV reverse transcriptase, the primer GACTCGAGTCGACATCGATTTTTTTTTTTTTTT and the procedure

of Maniatis (*Molecular Cloning* Cold Spring Harbor Laboratory (1982)). The human B2 receptor cDNA was selectively amplified using nested PCR. The first round PCR used the two primers CTCCGAGGAGGGGTGGG and CCTGAAAAGCAACTGTCCC and Taq DNA polymerase (Promega). Twenty-five rounds of PCR were done using the following conditions: 94°C, 1 minute for denaturation, 50°C, 1 minute for annealing followed by 72°C, 3 minutes for extension. Excess primers were removed with a Centricon 30 miniconcentrator. A portion of this first round reaction was used as a template in a second round of PCR using the following primers GCGAAGCTTCGTGAGGACTCCGTGCCC and CGCTCTAGACAAATTCACAGCCC. The number of rounds of PCR and the conditions were the same as those used for the first round. The DNA obtained after this second round was digested with the restriction enzymes Hind III and Xba I using standard methodology. Cesium chloride-purified pRc/CMV (Invitrogen) was also digested with Hind III and Xba I using standard methodology. The products of the two digests were resolved on a 1% low melt agarose gel. The human B2 receptor DNA (approximately 1.1 kb) and the pRc/CMV DNA (approximately 5.5 kb) were excised from the gel. The gel slices containing these DNAs were heated at 65°C and aliquots combined in a reaction containing T4 DNA ligase. The reaction was incubated overnight at 15°C.

An aliquot of this reaction was used to transform frozen competent *E. coli* DH5α cells (GibcoBRL). Transformants containing the human B2 receptor DNA were selected on LB + amp plates. One of the transformants was selected and the sequence of the human B2 receptor DNA insert determined using the

Sequenase enzyme (United States Biochemical) according to the manufacturer's instructions. This sequence was compared to the sequence of Hess et al (*Biochemical and Biophysical Research Communications* 184:260 1992)). Several nucleotide misincorporations were detected and those that altered the amino acid sequence of the receptor were corrected using site-directed mutagenesis (Kunkel et al *Methods in Enzymology* 154:367 (1987)). The human BK2 receptor-pRc/CMV plasmid was transfected into CHO-k1 cells using the Lipofectamine reagent (GibcoBRL). Transfectants were selected with the antibiotic G418 and screened for ^{3}H-bradykinin (Dupont NEN) binding. One clone, S34f, was chosen based upon binding levels, binding kinetics and inhibition patterns as the clone to be used for all human B2 receptor binding assays.

Cells containing the receptor were grown to confluence and harvested by scraping into PBS and homogenized. Membranes were prepared by centrifugation in 25mM TES pH6.8 containing 1mM 1.10 Phenanthroline. The final pellet was resuspended in assay buffer (25 mM TES, 1mM 1.10 Phenanthroline, 1mM DTT, 2M Captopril, 0.1% BSA pH=6.8) and frozen. In binding experiments, 60 μg membranes were incubated in 315 μl of assay buffer with 0.3 nM 3H-Bradykinin and various amounts of test compound. Incubation was carried out at room temperature for 45 min. The assay was terminated by filtration through a polyethylenimine soaked G-A filter. Radioactivity was measured on a Wallac 1450 MicroBeta LSC.

The K_d for the receptor was determined by kinetic analysis of on and off rates utilizing a single exponential decay off rate. K_i's for compounds were calculated using the following equation:

$K_i = IC_{50}/(1+[L]/K_d)$, where L=free ligand concentration. For comparison, the Ki for bradykinin was 10.4 ± .3.

Compound	Human Receptor Clone B2 receptor binding	n
1	15.2	1
2	10.35	2
3	11.85	2
5	10.16	2
7	14.1	1
15	13.95	2
16	9.51	2
22	10.9	2
23	10.8	3
24	12.25	3
25	12.9	1
27	9.75	2
28	12.83	2
30	12.2	2
31	11	2
45	9.85	2
47	10.26	2
53	10.85	2
56	9.4	2
60	10.16	2
61	8.39	2

EXAMPLE XV - Dog Blood Pressure

Mongrel dogs of either sex, weight range 10-15 kg were used. These were anaesthetized with pentobarbital, 30mg/kg i.v., and catheters were placed in one femoral artery and both femoral veins for the recording of blood pressure and infusion and injection

of compounds. Responses to BK (1nM) and des-Arg^9-BK (50nM) were produced in the absence and presence of antagonist as described above for the rabbit, and the ED_{50} was calculated. After the end of the infusion of the highest dose of compound 1 or 7 (1μg/kg/min), BK and des-Arg_9-BK were injected at intervals for up to four hours.

BLOOD PRESSURE DATA

Compound	Dog	
	B2	B1
1	0.03	0.24
7	0.17	0.35

Mean ED_{50} values in mg/kg/min for compounds 1 and 7 against B2 and B1 receptors-mediated hypotensive responses in the dog. (n=3).

Duration of action: For the dog, following a 25 minute infusion of 1 mg/kg/min of compound 7 responses to BK and des-Arg^9-Bk were normal 60 minutes later. On the other hand, after compound 1, responses to both kinins were still 100% blocked at 4 hours.

SEQUENCE LISTING

1) GENERAL INFORMATION:

(i) APPLICANT: STEWART, JOHN M.
GERA, LAJOS
WHALLEY, ERIC

(ii) TITLE OF INVENTION: BRADYKININ ANTAGONIST PEPTIDES CONTAINING INDANE-SUBSTITUTED AMINO ACIDS

(iii) NUMBER OF SEQUENCES: 19

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: CUSHMAN DARBY & CUSHMAN, L.L.P.
(B) STREET: 1100 New York Avenue, N.W.
(C) CITY: Washington
(D) STATE: D.C.
(E) COUNTRY: U.S.A.
(F) ZIP: 20005-3918

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/344,636
(B) FILING DATE: 18-NOV-1994
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: KOKULIS, PAUL N.
(B) REGISTRATION NUMBER: 16,773
(C) REFERENCE/DOCKET NUMBER: 216471/DKT. 19

(ix) TELECOMMUNICATION INFORMATION:
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(C) TELEX: 6714627 CUSH

2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Xaa Arg Pro Xaa Gly Xaa Ser Xaa Xaa Arg
1 5 10

) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Xaa Arg Pro Xaa Gly Xaa Ser Xaa Xaa Arg
1 5 10

) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Arg Pro Pro Gly Phe Ser Pro Phe Arg
1 5

) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Arg Pro Xaa Gly Xaa Ser Xaa Xaa Arg
1 5

) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Xaa Xaa Arg Pro Xaa Gly Xaa Ser Xaa Xaa Arg
1 5 10

) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Xaa Xaa Arg Pro Xaa Gly Phe Ser Xaa Xaa Arg
1 5 10

) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Xaa Arg Pro Xaa Gly Xaa Lys Xaa Xaa Arg
1 5 10

-) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Xaa Arg Pro Xaa Gly Xaa Ser Xaa Tyr Arg
1 5 10

:) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Xaa Arg Pro Xaa Gly His Ser Xaa Xaa Arg
1 5 10

:) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Arg Arg Pro Xaa Gly Xaa Ser Xaa Xaa Arg
1 5 10

INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Xaa Xaa Arg Pro Xaa Gly Xaa Lys Xaa Xaa Arg
1 5 10

2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Xaa Xaa Arg Pro Xaa Gly Xaa Thr Xaa Xaa Arg
1 5 10

2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Xaa Xaa Arg Pro Xaa Gly Xaa Glu Xaa Xaa Arg
1 5 10

2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Xaa Xaa Arg Pro Xaa Gly His Ser Xaa Xaa Arg
1 5 10

2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Xaa Arg Arg Pro Xaa Gly Xaa Ser Xaa Xaa Arg
1 5 10

2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Xaa Xaa Arg Pro Xaa Gly Xaa Ser Xaa Trp Arg
1 5 10

2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Xaa Xaa Arg Pro Xaa Gly Xaa Lys Xaa Xaa Arg
1 5 10

:) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Xaa Arg Arg Pro Xaa Gly Xaa Ser Xaa Xaa Arg
1 5 10

:) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Xaa Xaa Arg Pro Xaa Gly Xaa Ser Xaa Tyr Arg
1 5 10

WHAT IS CLAIMED IS:

1. A bradykinin antagonist according to the formula:

$$X--A^0--B^1--C^2--D^3--E^4--F^5--G^6--H^7--J^8--K^9--Z$$

wherein

X is optionally absent but if present is an aromatic, aliphatic, aromatic-aliphatic, alicyclic, heterocyclic or urethane-type acylating group, a guanidyl group, or at least one amino acid;

A^0, B^1, C^2, D^3, E^4, and K^9 are basic or neutral aromatic, aliphatic, heterocyclic, or alicyclic amino acids;

G^6 is an aromatic, aliphatic, heterocyclic, or alicyclic amino acid;

Z is optionally absent but if present is at least one amino acid; and

at least one of the following is true:

F^5 is Igla, Iglb, Niga, or Nigb;

H^7 is D-Igla, D-Iglb, Niga, or Nigb; or

J^8 is Igla, Iglb, Niga, or Nigb;

wherein if F^5 is not one of the above, it is an aromatic, aliphatic, aliphatic heterocyclic, or alicyclic amino acid,

if H^7 is not one of the above it is an aromatic, aliphatic, aliphatic heterocyclic, or alicyclic amino acid of the D configuration, and

if J^8 is not one of the above, it is an aromatic, aliphatic, aliphatic heterocyclic, or alicyclic amino acid.

2. The bradykinin antagonist peptide according to claim 1, wherein

A^0 and B^1 are basic amino acids;

C^2 and D^3 are Pro;
E^4 is Gly;
F^5 is an Indanyl amino acid;
G^6 is Ser;
H^7 is a D-Indanyl amino acid;
J^8 is an Indanyl amino acid; and
K^9 is a basic amino acid.

3. The bradykinin antagonist according to claim 1, wherein
X is Aaa, Aca, Acetyl, Dhq, Nba, Tba, Cha, Gun, or Cpa;
A^0 is DArg, DLys, Arg or Lys;
B^1 is DArg, DLys, Arg or Lys;
C^2 is Pro, DMF, NMF, MPIV, Hyp, Azt, Dhp, Inip, Thz, or Pop;
D^3 is Hyp, Pop, Niga, MPIV, Pro, Azt, Dhp, Inip, or Thz;
E^4 is Gly, Iglb, Nigb, Ala, or Gly;
F^5 is Igla, Ser(SO), Niga, Nigb, Leu, Chg, Ile, Val, Alg, Oic, Pop, Nle, or DMF;
G^6 is Ser, DIglb, HBQ, Cys, or Gly
H^7 is DIgla, Iglb, Niga, Nigb, DLeu, DChg, DIle, DVal, DCpg, DOic, DPop, DNle or DDMF;
J^8 is Igla, Lys, Niga, Nigb, Leu, Chg, Ile, Val, Cpg, Oic, Pop, or Nle; and
K^9 is Arg or DArg.

4. The bradykinin antagonist according to claim 1, wherein
F^5 is Phe, Thi, Cpg, or Chg;

5. The bradykinin antagonist according to claim 1 , wherein
H^7 is D-Tic, D-Cpg, or D-Chg.

6. The bradykinin antagonist according to claim 1, wherein

J^8 is Tic, Nbn, Oic, Cpg, or Chg.

7. The bradykinin antagonist according to claim 3, wherein

A^0 is D-Arg;
B^1 is Arg;
C^2 is Pro or Hyp;
D^3 is Pro or Hyp;
E^4 is Gly;
G^6 is Ser, Ser(SO), or HBQ; and
K^9 is Arg.

8. The bradykinin antagonist according to claim 2, of the formula

H-DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Iglb-Arg.

9. According to claim 1, of the formula

DArg-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Oic-Arg
DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIglb-Oic-Arg.
Gun-Arg-Pro-Hyp-Gly-Iglb-Ser-DIglb-Oic-Arg or
Gun-Arg-Pro-Hyp-Gly-Thi-Ser-DIglb-Oic-Arg

10. A method of antagonizing B1 receptors *in vivo* in a host in need of such antagonist by administering to said host the compound according to claim 1 with a pharmaceutically acceptable carrier in an amount effective to antagonize V1 reception.

11. A method of antagonizing B2 receptors *in vivo* in a host in need of such antagonist by administering to said host the compound according to claim 1 with a

pharmaceutically acceptable carrier in an amount effective to antagonize B2 receptors.

12. A method of treating or ameliorating disease conditions caused by induction of B1 and/or B2 receptors by administering to a host in need of such antagonism the compound according to claim 1 with a pharmaceutically acceptable carrier in an amount effective to antagonize B1 and/or B2 receptors.

13. A method of treating or preventing pain or inflammation by administering to a host in need of such treatment or prevention, a therapeutically effective amount of the compound according to claim 1 with a pharmaceutically acceptable carrier.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US 95/15080

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9524422	14-09-95	AU-B- 1932595	25-09-95

INTERNATIONAL SEARCH REPORT

Inter onal application No.
PCT/US95/15080

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 10-14
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although these claims refer to a method of treatment of the human body, the search has been carried out and based on the alleged effects of the products.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Internation. ,plication No
PCT/US 95/15080

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PEPTIDES, CHEMISTRY, STRUCTURE AND BIOLOGY. PROC. XIII AM. PEPT. SYMP., JUNE 20-25, 1993, EDMONTON, CANADA, 1994 ESCOM, LEIDEN, pages 449-451, L GERA ET AL. 'Bradykinin antagonists containing unusual amino acids ahow long-lasting action in vivo' see the whole document	1-14
A	JOURNAL OF MEDICINAL CHEMISTRY, vol. 37, no. 11, 27 May 1994 WASHINGTON US, pages 1586-1601, H JOSIEN ET AL. 'Design and synthesis of side-chain conformationally restricted phenylalanines and their use for structure-activity studies on tachykinin NK-1 receptor' see the whole document	1
A	CHEMICAL ABSTRACTS, vol. 119, no. 15, 11 October 1993 Columbus, Ohio, US; abstract no. 160844g, T NAKATSUKA ET AL. 'Preparation of 2-(1-indanyl)glycine and related compounds' page 999; see abstract & JP,A,00 585 999 (SUNTORY LTD.) 6 April 1993	1
P,X	WO,A,95 24422 (CORTECH, INC.) 14 September 1995 see the whole documents, in particular the example 4	1-14

INTERNATIONAL SEARCH REPORT

International application No
PCT/US 95/15080

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K7/18 A61K38/08

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JOURNAL OF MEDICINAL CHEMISTRY, vol. 34, no. 8, August 1991 WASHINGTON US, page 2649-2653 D J KYLE ET AL. 'Probing the bradykinin receptor; mapping of the geometric totpography using ethers of hydroxyproline in novel peptides' see the whole document --- -/--	1-14

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 April 1996	23. 04. 96

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,	Masturzo, P